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Histochemical detection of expression of binding sites for labelled hyaluronic acid and carrier-immobilized synthetic (histo-blood group trisaccharides) or biochemically purified (ganglioside GM_1) glycoligands in nasal polyps and other human lesions including neoplasms

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Summary. This study is intended to demonstrate the versatility and feasibility of custom-made oligosaccharide-exposing neoglycoconjugates including histo-blood group epitopes in various human lesions, including nasal polyps. The binding of the biotinylated probes was determined on formalin-fixed paraffin-embedded sections from archive materials. The general aspects of our results may be interpreted as follows: the neoglycoconjugates used here can readily detect differences in the ability of cells to bind glycan residues in tissue sections, thereby enabling the extent of the binding capacity of various types of human lesions to be compared. Furthermore, the reactivity to glycan may reflect characteristics of the cells and their environment.

The investigation into pathological disorders with respect to the binding capacity of these carrier-immobilized mono- or oligosaccharide structures derived from custom-made synthesis or biochemical purification is based on the prospect of translating progress in this field into the establishment of potentially beneficial procedures for medical diagnosis and pathological classification.

Key words: Neoglycoconjugates, Glycohistochemistry, Nasal polyps, Neoplasms

Introduction

Nasal polyps are usually considered as a single entity (Drake-Lee, 1993). However, for some authors they are probably a manifestation of different clinical and aetio-pathogenetic entities, and further identification of such subtypes is needed to improve treatment strategy (Larsen and Tos, 1991; Davidson and Hellquist, 1993). Nasal polyps often occur in association with numerous other diseases of the upper respiratory tract, e.g. rhinitis (Mullarkey et al., 1980), asthma (Settipane and Chafee, 1977), cystic fibrosis (Schwachman et al., 1962), aspirin idiosyncracy (Settipane, 1986) and Kartagener's syndrome (Imbrie, 1981).

Many authors have favoured allergy as the underlying cause of this pathological process which they relate to the infiltration of inflammatory cells, of which eosinophils are prominent (Bunnag et al., 1983). However, allergic diseases are no more common in patients with polyps than in the normal population, and allergy is not the only cause of nasal polyps (Davidson and Hellquist, 1993; Drake-Lee, 1993).

Until now all attempts at correlating clinical and histological data have been fruitless. New approaches and therefore called for. Recently, some authors studied the expression of cell adhesion molecules (VCAM, ICAM, selectins) (Jahnsen et al., 1995). The characterization of the oligosaccharide residues of glycoconjugates is another potentially valuable method of research. Analysis of the presence of glycan epitopes in tissue sections is a salient step in the delineation of their biological significance. The structures of these sugar residues of glycoconjugates have been found to be altered in association with the disease process (Feizi, 1985; Hakomori, 1989; Dennis, 1992; Dwek, 1995). Carbohydrate-binding proteins such as lectins are widely employed as probes to appraise these changes (Raedler and Raedler, 1985; Caselitz, 1987; Damjanov, 1987; Alroy et al., 1988; Danguy et al., 1988, 1994, 1996; Walker, 1989; Gabius and Gabius, 1991, 1993; Spicer and Schulte, 1992; Danguy, 1995; Salmon et al., 1996).

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The characterization of the endogenous sugar receptors (endolectins) in tissues as the protein part in the recognitive glycan-protein interactions opens a perspective to diligently analyze the ligand properties of any reactive carbohydrate sequence (Gabius and Bardosi, 1991; Gabius et al., 1993; Danguy, 1995; Danguy et al., 1995, 1996; Kannan and Nair, 1996).

The aim of the present study is to determine the expression of binding sites for a distinct set of glycostructures (directly labelled glycan in the case of hyaluronic acid, biochemically purified and chemically conjugated lysoganglioside GM_1 and synthetic di- and trisaccharides) by a routine histochemical procedure using sections of specimens of nasal polyps with biotinylated markers as laboratory tools. Moreover, we assess the versatility and the feasibility of this histochemical ligand approach by comparing this pathological nasal process with other lesions.

Materials and methods

Patients

Nasal polyps were obtained from 9 patients who required surgical treatment for nasal obstruction. The other cases included in this study comprised urinary bladder tumor tissue, breast tissue, carcinomas of the colon, superficial spreading type melanomas, prostatic hyperplasia and glioblastomas from archive material, i.e. paraffin-embedded formalin-fixed tissues belonging to the Department of Pathology of the Erasmus Hospital (Brussels, Belgium). These specimens were from patients who had undergone surgery. Three specimens were studied for each pathology.

Ligands

The applied ligands included synthetic or biochemically carbohydrate structures, i.e. galactose ß1,3,N-acetylgalactosamine (Galß1,3GalNAc-BSA-biotin, T-antigen), N-acetylneuraminic acid (NANA-BSA-biotin); Galß1,3GalNAcß1,4(NeuAcα2-3)Galß1,4Glcß1,1 ceramide (GM_1-BSA-biotin) and histoblood group A, B and H trisaccharides, attached either to bovine serum albumin or to a defined polyacrylamide matrix, as well as biotinylated hyaluronic acid (HA). The techniques for carrier preparation, their biotinylation and efficient conjugation of the ligands are described in detail elsewhere (Gabius and Bardosi, 1991; Gabius et al., 1990a-c, 1993; Abramenko et al., 1992; Kayser et al., 1994). Hyaluronic acid was labelled by subsequent reaction steps involving adipic dihydrazide and sulfo-NHS-biotin, as described (Pouyani and Prestwick, 1994).

Glycohistochemical processing

The ligands were applied to formalin-fixed, paraffin-embedded tissue which had been cut into histological sections 5 μm thick. The sections were dewaxed in toluene and rehydrated in a graded alcohol series. Some sections were stained with haematoxylin and eosin for the visualization of the general histology. The endogenous peroxidase activity was blocked with 0.3% methanolic hydrogen peroxide solution for 15 min. Non-specific binding sites were saturated in a further incubation step with 0.1% carbohydrate-free bovine serum albumin (BSA) in 0.1M phosphate buffer solutions (PBS) for 30 min as previously described (Kayser et al., 1994; Danguy et al., 1995).

Biotinylated markers were applied to sections for 30 min at room temperature at a concentration of 50 μg/ml. After washing in PBS buffer to remove unbound probe, the specific binding of the individual markers was visualized by the application of the avidin/biotin complex (ABC Elite kit, Vector Labs, Burlingame, CA) and the subsequent enzymatic development of the coloured product from the chromogenic substrates diaminobenzidine and hydrogen peroxide (Kayser et al., 1994; Danguy et al., 1995). As a final step, counterstaining with haematoxylin and mounting were performed. The parallel performance of standard positive and negative controls, as described in detail elsewhere (Akif et al., 1994, 1995; Danguy et al., 1995), ascertained the specificity of the binding reactions.

Semiquantitative assessments of the glycohistochemical staining

The tumor specimens were examined independently by three researchers. Two parameters were taken into account, namely the binding intensity index and the percentage of cells in the individual sample reacting with the neoglycoconjugates. The tumours were also divided into four classes based on the percentage of binding: no labelling (0), 1 to 33% of the tumours labelled (1), 34 to 66% of the tumours labelled (2), >66% of the tumours labelled (3). The degree of cytoplasmic intensity was evaluated as negative (0), weak or doubtful (1), medium (2) and strong (3). The following possible values were thus obtained: 0-11-12-13-21-22-23-31-32-33 (see Tables 1, 2), with the first value describing the spatial extent of tissue labelling and the second its intensity.

Results

Tables 1, 2 give the detailed pattern of the membrane and cytoplasmic staining with respect to the percentage of positive cells in the total cell population and also the staining intensity for each lesion. The histochemical expression of some neoglycoconjugates is illustrated in Figs. 1-3. The tables show that within the same case the percentage of cells which express specific ligand-binding sites for the tested probes often varied, as did the staining intensity. This ligand-dependent heterogeneity is an indication for the inherent specificity of binding to the probes with evidently different sugar structures.

Table 1. Expression of binding sites for T-antigen (T), hyaluronic acid (HA) and histoblood group A (A), B (B) and H (H) oligosaccharides, lysoganglioside GM_1 (GM_1), and N-acetylneuraminic acid (NANA) to various lesions.

ANATOMICAL SITE AND TISSUE TYPE	PATIENT No.	TYPE OF MARKER						
		T	HA	A	B	H	GM_1	NANA
Bladder								
Transitional cell carcinoma Gr 2	1	0	0	0	0	0	11	11
Transitional cell carcinoma Gr 2	2	0	0	0	0	0	11	11
Transitional cell carcinoma Gr 2	3	0	0	0	0	0	32	21
Brain								
Glioblastoma Gr 4	4	23	11	21	11	11	23	23
Glioblastoma Gr 4	5	22	11	22	11	11	32	32
Glioblastoma Gr 4	6	23	0	11	0	11	33	23
Breast								
Invasive ductal carcinoma (NOS) Gr 2	7	31	11	0	0	11	33	33
Invasive ductal carcinoma Gr 3	8	21	11	21	0	0	33	33
Fibroadenoma	9	22	11	0	11	0	32	22
Colon								
Adenocarcinoma Dukes'C	10	21	11	11	0	11	23	33
Adenocarcinoma Dukes'C	11	22	0	0	0	11	33	33
Adenocarcinoma Dukes'C	12	21	0	0	0	11	33	33
Skin								
Superficial spreading melanoma (SSM)	13	12	12	12	12	12	12	12
Superficial spreading melanoma (SSM)	14	12	0	0	0	0	32	22
Prostate								
Benign hyperplasia (BPH)	15	32	21	12	32	33	33	33
Benign hyperplasia (BPH)	16	32	11	31	31	32	33	33
Benign hyperplasia (BPH)	17	32	11	21	31	32	33	33

The first number represent the percentage of positive cells whereas the second one represents the intensity of labelling, as is detailed under Materials and methods.

Table 2. Expression of binding sites for T-antigen (T), hyaluronic acid (HA) and histoblood group A (A), B (B) and H (H) oligosaccharides, lysoganglioside GM_1 (GM_1), and N-acetylneuraminic acid (NANA) to nasal polyps.

CASE		TYPE OF MARKER						
		T	HA	A	B	H	GM_1	NANA
1	Surface epithelium	33	33	33	33	33	33	33
	Glandular epithelium	33	0	13	0	21	33	11
2	Surface epithelium	13	11	12	11	12	12	22
	Glandular epithelium	0	0	11	11	0	11	11
3	Surface epithelium	11	12	13	23	23	23	13
	Glandular epithelium	0	0	11	21	21	13	23
4	Surface epithelium	12	11	12	11	11	13	11
	Glandular epithelium	0	0	12	11	0	0	0
5	Surface epithelium	22	11	22	12	0	21	12
	Glandular epithelium	22	0	21	21	/	32	22
6	Surface epithelium	13	11	12	11	11	11	11
	Glandular epithelium	0	11	0	0	0	11	11
7	Surface epithelium	11	11	0	11	13	12	0
	Glandular epithelium	11	11	11	21	11	12	21
8	Surface epithelium	11	0	0	0	22	23	12
	Glandular epithelium	/	/	/	/	/	/	/
9	Surface epithelum	0	0	0	0	0	21	11
	Glandular epithelum	/	/	/	/	/	/	/

The first number represent the percentage of positive cells whereas the second one represents the intensity of labelling, as is detailed under Materials and methods. /: structure not present.

Both the GM_1-containing probe and the neoglycoprotein exposing N-acetylneuraminic acid as ligand, but not the also negatively charged hyaluronic acid caused heavy staining in all the fixed and paraffin-embedded tissue sections with the exception of two cases of transitional bladder cell carcinomas and some nasal polyps.

All the tissue sections with the exception of the bladder cell carcinomas and one case of nasal polyps were found to react positively to the T-antigen-bearing neoglycoprotein, and sometimes strong labelling was disclosed (glioblastomas, benign prostate hyperplasias (BPH)). Nuclear binding of the superficial and glandular epithelia was detected with GM_1-BSA-biotin and the T-antigen-bearing neoglycoprotein.

The presence of hyaluronic acid-binding sites in the processed specimens was rare except in one case of superficial spreading melanoma, one of BPH and two of nasal polyps. Concerning the histo-blood group trisaccharides, only the BPH exhibited a strong reactivity to the three probes. Transitional cell carcinoma specimens of the urinary bladder were negative with these three markers. This neoplasm was either weakly stained, or not stained at all, by the ligands used (except

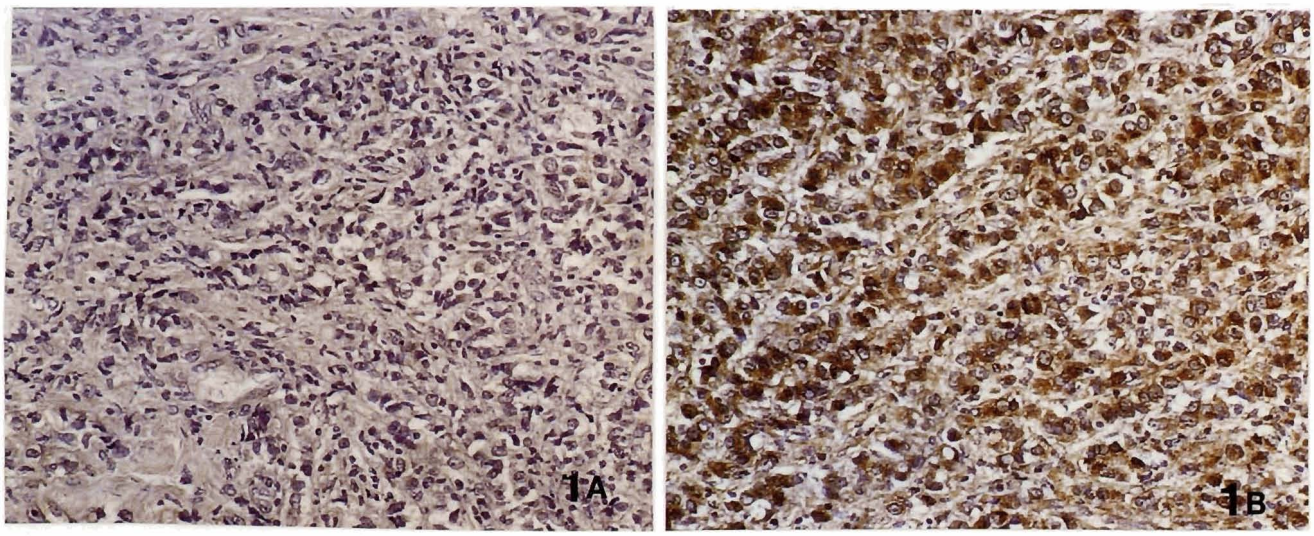

Fig. 1. Light micrographs of two serial sections of an invasive ductal carcinoma of the breast after application of biotinylated blood group H-trisaccharide (**A**) -exposing neoglycoconjugate and biotinylated N-acetylneuraminic acid-BSA (**B**), ABC reagents and the chromogen 3,3'-diamino-benzidine. Light counterstaining with haematoxylin. The cancer cells are not labelled with H-tri whereas they are strongly stained with SA. x 160

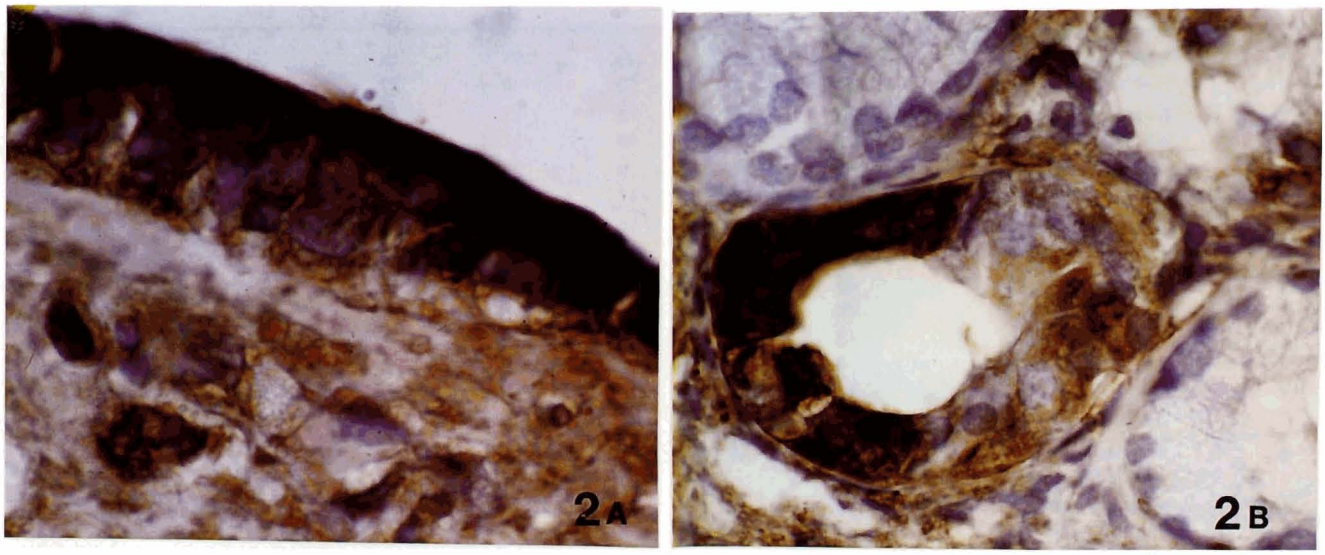

Fig. 2. Detection of specific binding for the biotinylated T antigen-bearing neoglycoprotein in nasal polyp, following incubation with ABC reagents and light counterstaining. The lining epithelium is strongly stained (**A**). The glandular epithelium discloses a heterogeneous binding pattern (**B**). x 1,024

for NANA-BSA and GM_1-BSA). The other lesions expressed variable reactivity.

One case of invasive grade 3 ductal carcinoma showed a moderate binding with the histoblood group A trisaccharide. Dissimilarities in ligand binding between the nine cases of nasal polyps were obvious. More particularly, the glandular tissue observed in most cases reacted differently when compared to the surface epithelium of the lesions and the same tissue from other patients.

Discussion

Using a well-defined panel of neoglycoproteins we analysed the expression of binding sites for selected carbohydrate structures in histological sections of nasal polyps and other lesions. The special interest in these probes stems from the fact that pathological aberrations are accompanied by alterations in carbohydrate structures (Feizi, 1985; Hakomori, 1989; Gabius and Gabius, 1991; Dwek, 1995).

Over the last few years increasing interest has been shown towards endogenous lectins (Gabius et al., 1993; Lee and Lee, 1994; Danguy et al., 1995) because these compounds have been demonstrated to be involved in a number of biological processes such as cell growth, intercell communications and biosignalling (Drickamer, 1988, 1994; Gabius, 1991; Zanetta et al., 1994). The staining reaction of a panel of biotinylated neoglycoproteins in paraffin-embedded specimen from various lesions provides evidence for differences in the expression of endogenous glycan receptors by different types of pathological tissues. Studies have found that endogenous lectins may be surface-exposed or localized in the cytoplasm and nucleus (Gabius, 1991). The presence of sugar receptors on tumor cell surfaces (Gabius et al., 1994) where they can influence tumor spread mechanisms has also been inferred.

A neoglycoprotein comprises a carrier, a chemical linker between the carrier and the carbohydrate group, and also the histochemically crucial glycan structure. Rigorous controls had to be used to exclude any contribution of the non-carbohydrate part of the molecule to the measured extent of the carbohydrate-dependent labelling (Gabius et al., 1993). In addition, it must be borne in mind that neoglycoproteins can bind only to sites that are accessible, to sites where their affinity is capable of displacing an endogenous ligand and which are not negatively affected by tissue processing. All these considerations having been taken into account, the present study demonstrates the presence of endogenous mono- or oligosaccharide-binding sites and different binding reactivities depending upon the anatomical location, emphasizing the versatility of the applied probes. By combining chemical synthesis or biochemical purification with histochemical application, the structural complexity to the carbohydrate structure is increased. As is shown in Tables 1 and 2, and illustrated in the figures, the availability of probes with such carbohydrate ligands enhances the versatility of neoglycoconjugates as potent lectin-localizing tools.

At present, although there is no definite proof of a particular function for any of the nuclear, cytoplasmic or membrane sugar receptors in tumor cells in vivo, it is conceivable that they may play some role in the

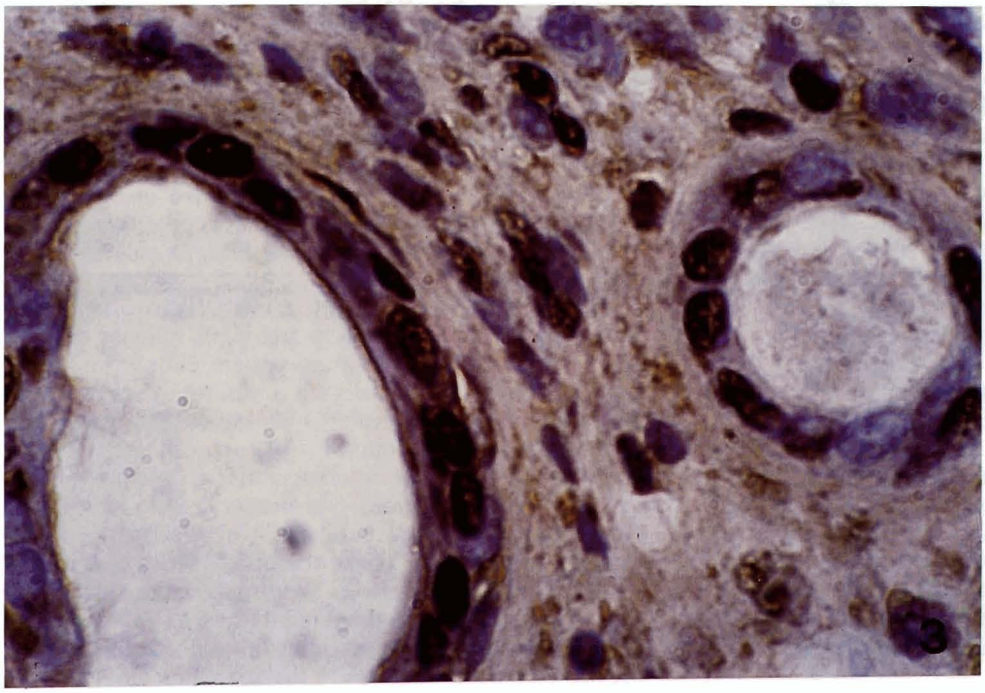

Fig. 3. Nasal polyp: glandular duct epithelium. Heterogeneous binding pattern with biotinylated GM_1-BSA. Some nuclei are strongly stained. x 1,024

regulation of gene expression, in recognitive interactions at certain stages of the cell cycle, in development, or in tumor growth and spread by specific interactions with cellular glycoconjugates of tumor cells or diverse types of host cells.

Most glycohistochemical studies have explored the distribution and modifications of carbohydrate sequences by means of lectin histochemistry in normal and pathological human tissues (Alroy et al., 1988; Figols et al., 1991; Danguy et al., 1994; Danguy, 1995; Salmon et al., 1996). The design and application of labelled neoglycoconjugates opens a new field in histopathology. Analyses in this field have revealed that the expression of glycoligand-binding sites is responsive to changes in the state of differentiation, cell atypia or microenvironment regulated in vitro by chemical inducers or modified oncogene expression (Gabius et al., 1993; Lee and Lee, 1994; Zanetta et al., 1994; Danguy et al., 1995). With respect to the practice of routine diagnosis, the use of neoglycoconjugates has already been fruitful (Bardosi et al., 1988; Gabius et al., 1988, 1990a,b,c; Kayser et al., 1989). As illustrated exemplarily in Fig. 3, the GM_1-containing probe caused significant staining in nearly all the fixed and paraffin-embedded sections from all the specimens studied. This staining had previously been demonstrated in lung tumors (Gabius et al., 1990c). Moreover, this neoligandoprotein invariably bound rather strongly compared to the other markers. Glycohistochemical studies with NANA-BSA-biotin provided evidence for the abundance of sialic acid-binding sites. The binding pattern of this probe is very similar to that of GM_1. This is not surprising because the lysoganglioside GM_1 contains a complex polar head made up of oligosaccharides containing one Neu5Ac residue.

In spite of the ubiquitous nature of sialic acids in mammals, relatively few reports substantiate the presence of specific endogenous receptors for these glycans. However, by means of covalently attached N-acetylneuraminic acid to a labeled carrier protein as a glycohistochemical probe, the presence of sialic acid-binding proteins in squamous head and neck cell carcinomas has been inferred (Steuer et al., 1995). When quiescent cells are stimulated, for example in oncogenesis, the activated chain of events leads to alteration of the level of expression of a multitude of molecules in the original cell. Among the diverse response on the transcriptional level stimulation of the G_0 fibroblasts led to expression of the calcyclin gene (Calabretta et al., 1986). Thus, histochemical localization of calcyclin in cells that respond to mitogenic stimulation is of interest. Calcyclin exhibits a notable affinity for sialic acids, namely N-glycol-neuraminic acid (Zeng and Gabius, 1991; Zeng et al., 1993). Therefore, the expression of binding sites for NANA-BSA is an indication of the presence of neuraminic acid-specific protein calcyclin. Functional implications for these molecules must await further experiments.

The expression of binding sites for A/H-blood group trisaccharides has been reported as correlating with patient survival in lung cancer (Kayser et al., 1994). In the present study, the detection of the capacity to recognize the A-, B- and H-trisaccharide epitopes as ligands is demonstrated in the majority of nasal polyps. Moreover, benign prostatic hyperplasia disclosed a relatively high level of binding for the three probes. Whether these endolectins serve as growth or motility control elements or as biological response modifiers, as suggested previously (Kayser et al., 1994), deserves further investigation.

Concerning the T-antigen-bearing neoglycoprotein, all the lesions expressed significant staining except the three transitional bladder cell carcinomas. In nasal polyps the surface epithelium of one specimen remained unlabelled and three specimens were weakly stained. Interestingly, Gabius et al. (1992) paid attention to the reactivity of the synthetically derived T-antigen as a ligand used for probing normal cases and cases with bladder lesions. They reported that nuclear staining in positive cases was enhanced by increasing histological grade. In the three cases studied here neither cytoplasmic nor nuclear staining was observed.

The secretion of hyaluronic acid from tumours to pleural fluid was described several years ago as a means of identifying mesotheliomas (Rasmussen and Faber, 1967). When comparing mesotheliomas and adeno-carcinomas of the lung, Chiu et al. (1984) reported a significantly higher concentration of HA in the mesotheliomas than in the adenocarcinomas. In primary ovarian carcinomas too, a high level of HA was also seen. This is of interest since these tumors are claimed to be derived from mesothelium and to produce HA. In our specimens the glycohistochemical assessment of HA receptors is difficult to interpret. The probe showed a moderate to strong binding to the epithelium of two nasal polyps, in one specimen of a superficial spreading melanoma and in one case of a benign prostate hyper-plasia. The other specimens were weakly labeled or not labeled at all. These results extend the application of labelled HA to detect respective receptors in ligand blot assays after SDS-PAGE, published recently (Yang et al., 1995; Yannasiello-Brown et al., 1996), to histochemical use.

Nasal polyps constitute a multifactorial disease which affects the nasal lining and sinus mucosa. Drake-Lee (1994) states that nasal polyps are not a disease, but merely a physical feature with a number of causes, and that polyps have a constant histological character. For other researchers, nasal polyps are probably a manifestation of different clinical and aetio-pathogenetic entities (see introduction), and further definition of clear-cut features of these entities is needed to improve treatment strategy. The variability observed in the staining pattern of various specimens of nasal polyps constitutes an encouragement for further research, the objective being to attempt delineation of correlations of the detected characteristics to the clinical behavior.

In conclusion, our findings of glycan receptors emphasize the often noted heterogeneity of abnormal cells in their expression of various determinants. This heterogeneity allows the definition of distinct subpopulations, possibly related to certain stages of development. The monitoring of the expression of glycan receptors and their in situ ligands as well as the correlation of these parameters to other cellular characteristics, for example to the integrated optical density or features of syntactic structure analysis (Kayser et al., 1995), is suggested to eventually contribute to the understanding of the functional relevance of the intratumoral heterogeneity.

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References

Abramenko T.V., Gluzman D.F., Korchagina E.Y., Zemlyanukhina I.V. and Bovin N.V. (1992). Oligosaccharide-binding molecules on the surface of human hematopoietic and lymphoid cells. FEBS Lett. 307, 283-286.

Akif F., Gabius H.-J. and Danguy A. (1994). Androgen status and expression of glycoconjugates and lectins in the epithelial cells of the mosue ventral prostate. A glycohistochemical approach. Histol. Histopathol. 9, 705-713.

Akif F., Gabius H.-J. and Danguy A. (1995). Estrous cycle-related alterations in the expression of glycoconjugates and lectins in the mouse endometrius shown histochemically. Tissue Cell 27, 197-206.

Alroy J., Ucci A. and Pereira M.E.A. (1988). Lectin histochemistry: an update. In: advances in immunohistochemistry. Delellis R.A. (ed). Raven Press. New York. pp 93-131.

Bardosi A., Dimitri T. and Gabius H.-J. (1988). Neoglycoproteins as tools in neuropathology: histochemical patterns of the extent of expression of endogenous carbohydrate-binding receptors like endogenous lectins. Virchows Arch. (B) 56, 35-43.

Bunnag C., Pacharee P., Vipulakom P. and Siryananda C. (1983). A study of allergic factor in nasal polyp patients. Ann. Allergy 50, 126-132.

Calabretta B., Battini R., Kaczmarek L., de Riel J.K. and Baserga R. (1986). Molecular clining of the cDNA for a growth factor-inducible gene with strong homology to S-100, a calcium-binding protein. J. Biol. Chem. 261, 12628-12632.

Caselitz J. (1987). Lectins and blood group substances as «tumor-markers». Curr. Top. Pathol. 77, 245-277.

Chiu B., Churg A., Tengblad A., Pearce R. and McCaughey W.T.E. (1984). Analysis of hyaluronic acid in the diagnosis of malignant mesothelioma. Cancer 54, 2195-2199.

Damjanov I. (1987). Biology of disease. Lectin cytochemistry and histochemistry. Lab. Invest. 57, 5-20.

Danguy A. (1995). Perspectives in modern glycohistochemistry. Eur. J. Histochem. 39, 5-14.

Danguy A., Kiss R. and Pasteels J.L. (1988). Lectins in histochemistry: a survey. Biol. Struct. Morphog. 1, 93-106.

Danguy A., Akif F., Pajak B. and Gabius H.-J. (1994). Contribution of carbohydrate histochemistry to glycobiology. Histol. Histopathol. 9, 155-171.

Danguy A., Kayser K., Bovin N.V. and Gabius H.-J. (1995). The relevance fo neoglycoconjugates for histology and pathology. Trends Glycosci. Glycotechnol. 7, 261-275.

Danguy A., Camby I., Salmon I. and Kiss R. (1996). Modern glycohistochemistry: a major contribution to morphological investigations. In: Glycosciences: status and perspectives. Gabius H.-J. and Gabius S. (eds). Chapman & Hall. Weinheim. (in press).

Davidson A. and Hellquist H.B. (1993). The so-called «Allergic» nasal polyp. ORL 55, 30-35.

Dennis J.W. (1992). Changes in glycosylation associated with malignant transformation and tumor progression. In: cell surface carbohydrates and cell development. Fukuda M. (ed). CRC Press. Boca Raton. pp 161-194.

Drake-Lee A.B. (1993). Nasal polyps. In: allergic and non-allergic rhinitis. Clinical aspects. Mygind M. and Naclerio R.M. (eds). Munksgaard. Copenhagen. pp 167-173.

Drake-Lee A.B. (1994). Medical treatment of nasal polyps. Rhinology 32, 1-4.

Drickamer K. (1988). Two distinct clases of carbohydrate-recognition domains in animal lectins. J. Biol. Chem. 263, 9557-9760.

Drickamer K. (1994). Molecular structure of animal lectins. In: Molecular glycobiology. Fukuda M. and Hindsgaul O. (eds). IRL Press. Oxford. pp 53-87.

Dwek R.A. (1995). Glycobiology: more functions for oligosaccharides. Science 269, 1234-1235.

Feizi T. (1985). Demonstration by monoclonal antibodies that carbohdyrate structures of glycoproteins and glycolipids are onco-developmental antigens. Nature 314, 53-57.

Figols G., Madrid J.F. and Cervos-Navarro J. (1991). Lectins as differentiation markers of human gliomas. Histol. Histopathol. 6, 79-85.

Gabius H.-J. (1991). Detection and functions of mammalian lectins - with emphasis on membrane lectins. Biochim. Biophys. Acta 1071, 1-18.

Gabius H.-J. and Bardosi A. (1991). Neoglycoproteins as tools in glycohistochemistry. Prog. Histochem. Cytochem. 22, 1-66.

Gabius H.-J. and Gabius S. (eds) (1991). Lectins and cancer. Springer-Verlag. Heidelberg, New York.

Gabius H.-J. and Gabius S. (eds). (1993). Lectins and glycobiology. Springer Verlag. Berlin, New York.

Gabius H.-J., Bodanowitz S. and Schauer A. (1988). Endogenous sugar-binding proteins in human breast tissue and benign and malignant breast lesions. Cancer 61, 1125-1131.

Gabius H.-J., Gabius S., Brinck U. and Schauer A. (1990a). Endogenous lectins with specificity to ß-galactosides and α- or ß-N-acetylgalactosaminides in human breast cancer. Their glycohistochemical detection in tissue sections by synthetically different types of neoglycoproteins, their quantitation on cultured cells by neoglycoenzymes and their usefulness as targets in lectin-mediated phototherapy in vitro. Pathol. Res. Pract. 186, 597-607.

Gabius H.-J., Schröter C., Gabius S., Brinck U. and Tietze L.F. (1990b). Binding of T-antigen-bearing neoglycoprotein and peanut agglutinin

to cultured tumor cells and breast carcinomas. J. Histochem. Cytochem. 38, 1625-1631.

Gabius S., Kayser K., Hellmann K.P., Ciesiolka T., Trittin A. and Gabius H.-J. (1990c). Carrier-immobilized derivatized lysoganglioside GM_1 is a ligand for specific binding sites in various human tumor cell types and peripheral blood lymphocytes and monocytes. Biochem. Biophys. Res. Commun. 169, 239-244.

Gabius H.-J., Bahn H., Holzhausen H.-J., Knolle J. and Stiller D. (1992). Neoglycoprotein binding to normal urothelium and grade-dependent changes in bladder lesions. Anticancer Res. 12, 987-992.

Gabius H.-J., Gabius S., Zemlyanukhina T.V., Bovin N.V., Brinck U., Danguy A., Joshi S.S., Kayser K., Schottelius J., Sinowatz F., Tietze L.F., Vidal-Vanaclocha F. and Zanetta J.-P. (1993). Reverse lectin histochemistry: design and application of glycoligands for detection fo cell and tissue lectins. Histol. Histopathol. 8, 369-383.

Gabius H.-J., André S., Danguy A., Kayser K. and Gabius S. (1994). Detection and quantification of carbohydrate-binding sites on cell surfaces and in tissue sections by neoglycoproteins. Meth. Enzymol. 242, 37-46.

Hakomori S. (1989). Aberrant glycosylation in tumors and tumor-associated carbohydrate antigens. Adv. Cancer Res. 52, 257-270.

Imbrie J.D. (1981). Kartagener's syndrome: a genetic defect affecting the function of cilia. Am. J. Oto-laryngol. 2, 215-222.

Jahnsen F.L., Haraldsen G., Aanesen J.P., Haye R. and Brandzaeg P. (1995). Eosinophil infiltration is related to increased expression of vascular cell adhesion molecule-1 in nasal polyps. Am. J. Respir. Cell Mol. Biol. 12, 624-632.

Kannan S. and Nair M.K. (1996). Lectins and neoglycoconjugates in histopathology. In: Glycosciences: status and perspectives. Gabius H.-J. and Gabius S. (eds). Chapman and Hall. Weinheim. (in press).

Kayser K., Heil M. and Gabius H.-J. (1989). Is the profile of binding of a panel of neoglycoproteins useful as a diagnostic marker in human lung cancer? Pathol. Res. Pract. 184, 621-629.

Kayser K., Bovin N.V., Korchagina E.Y., Zeilinger C., Zeng F.Y. and Gabius H.-J. (1994). Correlation of expression of binding sites for synthetic blood group A-, B- and H-trisaccharides and for sarcolectin with survival of patients with bronchial carcinoma. Eur. J. Cancer 30A, 653-657.

Kayser K., Bubenzer J., Kayser G., Eichorn S., Zemlyanukhina T., Bovin N.V., Andre S., Koopma J. and Gabius H.-J. (1995). Expression of lectin, interleukin-2 and histopathologic blood group binding sites in prostate cancer and its correlation with integrated optical density and syntactic structure analysis. Anal. Quant. Cytol. Histol. 17, 135-142.

Larsen P.L. and Tos M. (1991). Origin of nasal polyps. Laryngoscope 101, 305-312.

Lee Y.C. and Lee R.T. (eds). (1994). Neoglycoconjugates: preparations and applications. Academic Press. San Diego.

Mullarkey M.F., Hill J.S. and Webb D.R. (1980). Allergic and non-allergic rhinitis: their characterization with attention to the meaning of nasal eosinophilia. J. Allergy Clin. Immunol. 65, 122-126.

Pouyani T. and Prestwick G.D. (1994). Biotinylated hyaluronic acid: a new tool for probing hyaluronate-receptor interactions. Bioconjugate Chem. 5, 370-372.

Raedler A. and Raedler E. (1985). The use of lectins to study normal differentiation and malignant transformation. J. Cancer Res. Clin. Oncol. 109, 245-251.

Rasmussen K.N. and Faber V. (1967). Hyaluronic acid in 247 pleural fluids. Scand. J. Respir. Dis. 48. 366-371.

Salmon I., Camby I., Remmelink M., Rombaut K., Pasteels J.L., Kiss R. and Danguy A. (1996). Characterization of lectin histochemistry, ploidy level determination and proliferation index in meningo-theliomatous, fibroblastic and transitional meningiomas. Neuropathol. Appl. Neurobiol. 22, 68-76.

Settipane G.A. (1986). Asthma, aspirin intolerance and nasal polyps. New Engl. Reg. Allergy Proc. 7, 32-37.

Settipane G.A. and Chafee F.H. (1977). Nasal polyps in asthma and rhinitis. A review of 6.037 patients. J. Allergy Clin. Immunol. 59, 17-21.

Schwachman H., Kulczycki L.L., Mueller H.L. and Flake C.G. (1962). Nasal polypis in patients with cystic fibrosis. Pediatrics 30, 389-401.

Spicer S.S. and Schulte B.A. (1992). Diversity of cell glycoconjugates shown histochemically: a perspective. J. Histochem. Cytochem. 40, 1-38.

Steuer M.K., Steuer M., Bonkowsky V., Struz J., Gabius H.-J. and Hofstädter F. (1995). Will neoadjuvant chemotherapy affect the capacity of head and neck squamous cell carcinoma to bind neoglycoproteins? ORL 57, 22-27.

Walker R.A. (1989). The use of lectins in histopathology. Pathol. Res. Pract. 185, 826-835.

Yang B., Yang B.L. and Goetinck P.F. (1995). Biotinylated hyaluronic acid as a probe for identifying hyaluronic acid-binding proteins. Anal. Biochem. 228, 299-306.

Yannariello-Brown J., Zhou B., Ritchie D., Oka J.A. and Weigel P.H. (1996). A novel ligand blot assay detects different hyaluronan-binding proteins in rat liver hepatocytes and sinusoidal endothelial cells. Biochem. Biophys. Res. Commun. 218, 314-319.

Zanetta J.-P., Badache A., Maschke S., Marschal P. and Kuchler S. (1994). Carbohydrates and soluble lectins in the regulation of cell adhesion and proliferation. Histol. Histopathol. 9, 385-412.

Zeng F.Y. and Gabius H.-J. (1991). Carbohydrate-binding specificity of calcyclin and its expressin in human tissues and leukemic cells. Arch. Biochem. Biophys. 289, 137-144.

Zeng F.Y., Gerke V. and Gabius H.-J. (1993). Identification of annexin II, annexin VI and glyceraldehdye-3-phosphate dehydrogenase as calcyclin-binding proteins in bovine heart. Int. J. Biochem. 25, 1019-1027.

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